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## What Is Cyanmethemoglobin?

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Since previous workers<sup>1</sup> have explained the action of nitrites and methylene blue in cyanide poisoning as the formation of methemoglobin by these substances and the consequent union of CN with the methemoglobin to form cyanmethemoglobin, I was interested in finding out what constituted cyanmethemoglobin.

The literature is rather vague concerning this substance. The few brief descriptions are not quantitative and there seems to be a dispute as to its chemical configuration.<sup>2</sup>

I therefore studied the effects of either or both KCN and NaNO<sub>2</sub> on the spectrophotometric picture of oxyhemoglobin and reduced hemoglobin. The amount of NaNO<sub>2</sub> employed was sufficient to change 88% of the oxyhemoglobin to methemoglobin, and the chemically equivalent amount of KCN was used. The resulting hemoglobin derivatives were examined spectrophotometrically.

Oxyhemoglobin was formed by vigorous shaking either in air or oxygen; reduced hemoglobin by means of Stokes Reagent according to the usual procedure; methemoglobin, by adding NaNO<sub>2</sub>. *In vitro* experiments with sheep, rat and rabbit blood and *in vivo* experiments with rabbit and rat gave essentially similar results. The experiments here reported were done with sheep red blood cells *in vitro*. To each 2 cc. of defibrinated blood which had been washed several times and made up to original volume in 0.9% NaCl was added 0.2 cc. of 0.33 M NaNO<sub>2</sub> and/or KCN according to the experiment. For spectrophotometric examination 1 cc. of the resulting suspension was hemolyzed in 100 cc. of 0.4% NH<sub>4</sub>OH.

In no case did the absorption spectrum in the presence of KCN differ qualitatively from that of oxyhemoglobin. The ratio of the extinction coefficients at 540 and 560 m $\mu$ . was calculated and is given as R in Table I. This ratio indicates according to Ray, Blair and Thomas<sup>3</sup> the proportion of oxyhemoglobin in mixtures with reduced or methemoglobin.

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<sup>1</sup> Wendel, W. B., *J. Am. Med. Assoc.*, 1933, **100**, 1054; Hug, E., *Compt. Rend. Soc. Biol.*, 1933, **112**, 511.

<sup>2</sup> Hammarsten, O., *A Textbook of Physiological Chemistry*, 7th edition, 1914; Stadie, W. C., *J. Biol. Chem.*, 1920, **41**, 237.

<sup>3</sup> Ray, G. B., Blair, H. A., and Thomas, C. I., *J. Biol. Chem.*, 1932, **98**, 63.

TABLE I.

Form of hemoglobin	Value of R	% oxyhb.
Oxyhb. alone	1.63	100
Oxyhb. + KCN	1.62	
Methb. alone	1.28	12
Methb. + KCN	1.63	
Reduced Hb. alone	0.849	0
Reduced Hb. + KCN	1.62	

Table I shows that in the case of oxyhemoglobin alone the appropriate value of R was obtained. An identical absorption curve and value of R was obtained for oxyhemoglobin plus KCN. The methemoglobin preparation was found to contain 12% oxyhemoglobin (the remainder being methemoglobin), but when KCN was added to this, the absorption curve and R value immediately shifted to that characteristic of a pure oxyhemoglobin preparation. Similar results were obtained when KCN was added to reduced Hb. These readings were the same 2 hours later but 48 hours later the solutions containing  $\text{NaNO}_2$  gave the methemoglobin curve. These experiments were done at room temperature, but even when the blood cell suspensions containing KCN were warmed to  $40^\circ\text{C}$ . and were allowed to stand 10 minutes, the oxyhb. spectrum persisted.

It is tempting to conclude that since the cyanide containing suspensions gave the typical oxyhb. spectra, therefore the effect of the cyanide was to change all the hemoglobin to oxyhemoglobin and stabilize it. However, since the colors of the hemoglobin derivatives depend upon the configuration of the electrons around the Fe of the heme, it is conceivable that electron configurations could be set up which would be enough like those of oxyhemoglobin to give a spectrum indistinguishable from that of oxyhemoglobin. The observation of Cook<sup>4</sup> who states that when cyanide is added to oxyhemoglobin no  $\text{O}_2$  is evolved, is interesting in this connection. This may mean that the  $\text{O}_2$  is taken up by some other part of the molecule and therefore not released for measurement.

In conclusion it may be stated that no evidence for the existence of cyanmethemoglobin was found; that the absorption curve for cyanhemoglobin was identical with that for oxyhb., and that this was found to be the case regardless of whether the cyanide was added to oxyhemoglobin, or reduced hemoglobin, or methemoglobin.

<sup>4</sup> Cook, S. F., *J. Gen. Physiol.*, 1928, **11**, 339.